REMARKS

On page 3 of the Office Action, the Examiner rejects Claims 6-7, 20, 22-26, 28, 30-35, 37 and 39-53 under 35 U.S.C. § 103 as being unpatentable over Lyman et al in view of Elliot et al, Srivastava et al and Brem et al.

Specifically, the Examiner contends that Lyman et al teaches treating cancer by administering flt3-ligand in combination with another cytokine, e.g., GM-CSF. The Examiner notes that Lyman et al does not teach administration of a tumor antigen to the cancer patient to induce an immune response to the desired tumor antigen or that administration of flt3-ligand and/or GM-CSF will lead to an increase in the number of dendritic cells, as claimed. However, it is the Examiner's position that Elliot et al teaches vaccination of cancer patients with tumor antigens mixed with GM-CSF; and Srivastava et al teaches augmenting cancer vaccines comprising cancer cells and cancer antigens with cytokines, e.g., GM-CSF; and Brem et al teaches GM-CSF activates cytotoxic T lymphocytes which lead to the elimination of tumor cells by playing a role in the development of antigen processing cells, such as dendritic cells. Hence, the Examiner concludes that it would have been *prima facie* obvious to administer a tumor antigen with flt3-ligand and GM-CSF to treat cancer to achieve the present invention.

For the following reasons, Applicants respectfully traverse the Examiner's rejection.

None of the references cited by the Examiner teach or suggest, alone or in combination, use of flt3-ligand in an

amount sufficient to an increase in the number of dendritic cells, as claimed.

Lyman et al teaches a method for expanding hematopoietic stem and progenitor cells (see column 26, lines 23-37 thereof). The stem cells were cultured for 4 days (96 hours plus 24 hours with radioactive tag) and cultured an additional 2 days in the presence of flt3-ligand. This is an approximate culture time of 6 days for expanding hematopoietic stem and/or progenitor cells. In contrast, Examples 1-2 of the present application describe culture conditions for generating large numbers of dendritic cells. Example 1 describes culturing CD34+ cells in the presence of flt3-ligand for approximately 2 weeks and Example 3 shows administering flt3-ligand daily over a period of 19 days. Thus, the hematopoietic stem and/or progenitor cells must be exposed to flt3-ligand for an extended period in order to generate an increase in the number of dendritic cells in the patient. This feature is also not taught or suggested in any other reference cited by the Examiner.

In any event, in order to demonstrate that the administration of flt3-ligand in conjunction with GM-CSF and a tumor antigen provides unexpectedly superior results, in terms of preventing formation of tumors, over *inter* alia, administration of GM-CSF and a tumor antigen alone or Flt3-ligand and a tumor antigen, Applicants submit herewith a study carried out in mice. As shown in Table 1 thereof, tumors formed in 9 out of 10 mice when Flt3 ligand was administered with saline, and in 10 of out 10 mice when GM-CSF was administered with saline alone or in combination with Flt3-ligand. However, when Flt3-ligand and GM-CSF were

administered together with an antigen (BLP25), 4 out of 10 mice did not form tumors. This is in contrast to the situation when Flt3-ligand was administered together with an antigen (BLP25), where only 2 out of 10 mice did not form tumors or when GM-CSF was administered together with an antigen (BLP25), where only 1 out of 10 mice did not form tumors. Thus, the combination of Flt3-ligand, GM-CSF and tumor antigen provides unexpectedly superior results in terms of preventing formation of tumors.

Accordingly, Applicants respectfully submit that the present invention is not taught or suggested in Lyman et al and that the combination thereof with Elliot et al, Srivastava et al and Brem et al does not give rise to the present invention. In any event, Applicants data is sufficient to rebut any *prima facie* case of obviousness the Examiner might have raised. Thus, Applicants request withdrawal of the Examiner's rejection.

In view of the amendments to Claims and the arguments set forth above, reexamination, reconsideration and allowance are respectfully requested.

The Examiner is invited to contact the undersigned at the below-listed number on any matters which might arise.

Respectfully submitted,

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WASHINGTON OFFICE
23373
CUSTOMER NUMBER

Date: May 17, 2006

Aim of the study

This study was designed to test the anti-tumour effect of combination treatment with Flt3 ligand, pegylated GM-CSF and liposomal BLP25 vaccine in the MCA-38MUC1 mouse tumor model.

Test substances

		Lot#
BG-RG (L-BLP25)	BP1-148 400 ug/mL, lipid A adjuvant (200A-14) 200 ug/mL	BG99G27RG
BG-RJ (Ag liposomes)	BP1-148 0 ug/mL, lipid A (200A-14) 200 ug/mL	BG99G07RJ
Flt-3 ligand	Human, recombinant Flt-3 ligand produced in CHO cells	7772-018
Pegylated GM-CSF	Pegylated, murine GM-CSF	7283-44
Saline	Abbott Laboratories Ltd.	06-6945-2/R5

L-BLP25 is a liposomal formulation containing MUC1 based lipopeptide (BP1-148) and lipid A adjuvant.
Ag liposomes is a formulation containing a lipid A adjuvant only

Mice : C57Bl/6 human MUC1 female mice 13 wk old

Tumor : MCA-38MUC1 mouse colon carcinoma transfected with the human MUC1 gene.

Study design

Group	Mice	MCA-38MUC1	Flt-3 ligand	GM-CSF	L-BLP25
		Day 0 (Sept.20) s.c.	Day 0-11 (Sep.20-Oct.1) s.c. 10ug/100ul/dose	Day 7-11 (Sep.27-Oct.1) i.p. 2 ug/100ul/dose	Day 10, 17, 24 (Sept 30, Oct.7&14) s.c. 100 ug/250 ul/dose
1AB	10	+	+	-	Saline
2AB	10	+	-	+	Saline
3AB	10	+	+	+	Saline
4AB	10	+	+	-	BG-RG
5AB	10	+	-	+	BG-RG
6AB	10	+	+	+	BG-RG
7AB	10	+	+	+	BG-RJ
8AB	10	+	-	-	BG-RJ
9AB	10	+	-	-	BG-RG
10AB	10	+	-	-	Saline

Experimental procedure

Mice were challenged subcutaneously with 5x10c5 MCA-38MUC1 tumor cells and then randomized. Flt3 was injected s.c through 12 consecutive days starting on day 0, and pegylated GM-CSF was injected i.p. daily between day 7 and 11. Immunisations with BLP25 liposomal vaccine or Ag liposomes (no antigen, lipid A adjuvant only) (BG-RJ) were performed on day 10, 17 and 24. The tumour measurements were taken on a weekly basis, and tumour size was expressed as a product of bi-dimensional measures (mm2).

Results

As presented in Table 1 only the mice treated with both cytokines and BLP25 (group 6) or with both cytokines and Ag liposomes (group 7) showed high number of mice whose tumours showed no significant growth (tumour no take). If BLP25, Ag liposomes or both cytokines were administered separately no influence on tumour take was observed.

Table 1. Number of mice with or without tumour in each treated group.

Group	Treatment	Mice w/o tumour /total number per group
1	Flt-3 + saline	1/10
2	GM-CSF + saline	0/10
3	Flt-3 + GM-CSF + saline	0/10
4	Flt-3 + BLP25	2/10
5	GM-CSF + BLP25	1/10
6	Flt-3 + GM-CSF + BLP25	4/10
7	Flt-3 + GM-CSF + Ag liposomes	5/10
8	Ag liposomes	0/10
9	BLP25	0/10
10	Saline	0/10

The tumour growth kinetics are summarized in Figure 1 where all mice are included (with and without tumours) and Figure 2 where only the mice with tumours are included in the evaluation. The best synergism was observed when Ag liposomes were administered with Flt-3 and GM-CSF (group 7). The anti-tumour effect was statistically significant (statistics attached) when compared to Ag liposomes treatment (group 8) or both cytokine treatment (group 3) and to saline (group 10). Some synergism was observed (Fig.1) when mice were treated with BLP25 vaccine and both cytokines (group 6) as compared to BLP25 alone (group 9), but this observed improvement was not statistically significant.
On the other hand BLP25 alone produced a significant anti-tumour effect as compared to Ag liposomes group or saline group.

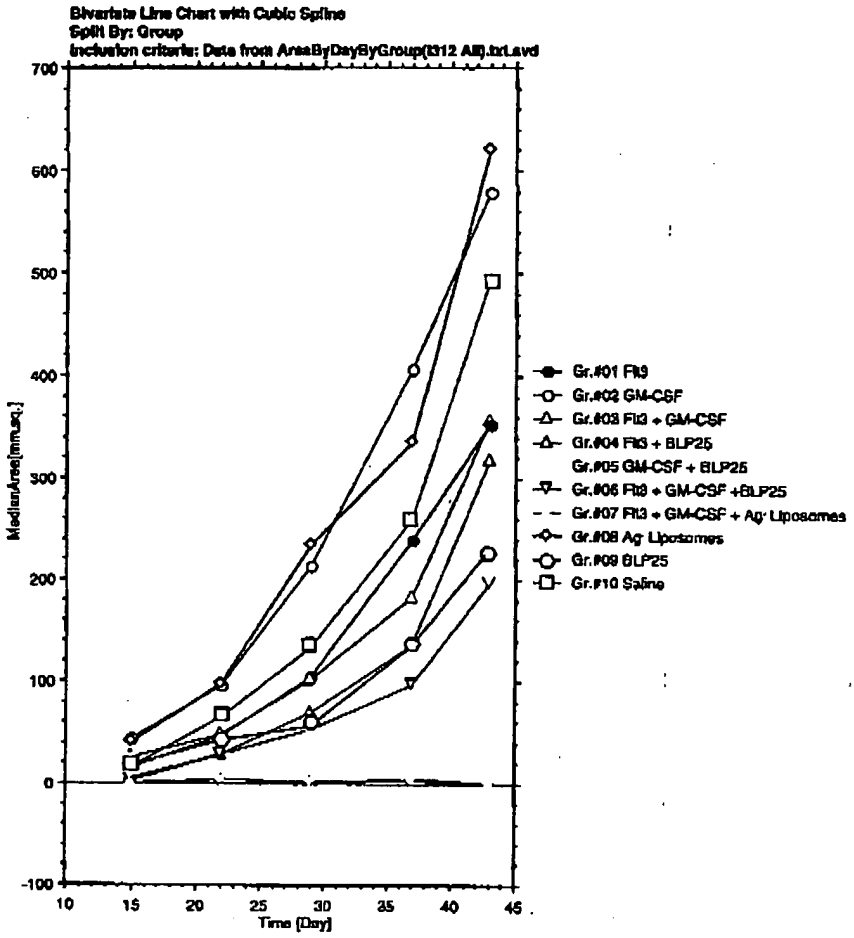

Fig 1. Tumour growth curves in mice challenged with MCA-38 MUC1 tumour and then treated with various combinations of Flt-3 ligand, pegylated GM-CSF, BLP25 liposomal vaccine or Ag liposomes. All mice were included into statistical evaluation (both with and without tumours).

Table 2. Statistically significant differences between mouse groups treated with various combinations of cytokines and BLP25 liposomal vaccine (only statistically significant groups are included).

Treatment	P-value
Flt-3 > saline	0.0377
Flt-3 > GM-CSF	0.0149
Flt-3 + GM-CSF + Ag liposomes > Flt-3	0.0175
Flt-3 > Ag liposomes	0.0147
Flt-3 + BLP25 > Saline	0.0095
GM-CSF + BLP25 > Saline	0.0042
Flt-3 + GM-CSF + BLP25 > Saline	0.0006
Flt-3 + GM-CSF + Ag liposomes > Saline	<.0001
BLP25 > Saline	0.0046
Flt-3 + BLP25 > GM-CSF	0.0035
GM-CSF + BLP25 > GM-CSF	0.0015
Flt-3+ GM-CSF + BLP25 > GM-CSF	0.0002
Flt-3 + GM-CSF + Ag liposomes > GM-CSF	<.0001
BLP25 > GM-CSF	0.0017
Flt-3 + GM-CSF + BLP25 > Flt-3 + GM-CSF	0.0324
Flt-3 + GM-CSF + Ag liposomes > Flt-3 + GM-CSF	0.0017
Flt-3 + GM-CSF + Ag liposomes > Flt-3 + BLP25	0.0470
Flt-3 + BLP25 > Ag liposomes	0.0044
GM-CSF + BLP25 > Ag liposomes	0.0021
Flt-3 + GM-CSF + BLP25 > Ag liposomes	0.0004
Flt-3 + GM-CSF + Ag liposomes > Ag liposomes	<.0001
BLP25 > Ag liposomes	0.0023

ANOVA Table for RankArea(Nov02)
Inclusion criteria: Protocol I312(All) from TumorData I312(All).svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
GroupIndex	9	23164.111	2573.790	5.587	<.0001	50.283	1.000
Residual	80	36853.678	460.671				

All groups statistical analysis (mice with and without tumours)

Fisher's PLSD for RankArea(Nov02)
Effect: GroupIndex
Significance Level: 5 %
Inclusion criteria: Protocol I312(All) from TumorData I312(All).svd

	Mean Dif.	Crit. Dif.	P-Value	
1, 10	-20.844	19.625	.0377	S
1, 2	-25.944	20.755	.0149	S
1, 3	-8.222	20.135	.4188	
1, 4	4.656	19.625	.6382	
1, 5	7.456	19.625	.4519	
1, 6	13.256	19.625	.1827	
1, 7	24.556	20.135	.0175	S
1, 8	-29.844	23.824	.0147	S
1, 9	7.156	19.625	.4702	
10, 2	-5.100	20.261	.6178	
10, 3	12.622	19.625	.2043	
10, 4	25.500	19.102	.0095	S
10, 5	28.300	19.102	.0042	S
10, 6	34.100	19.102	.0006	S
10, 7	45.400	19.625	<.0001	S
10, 8	-9.000	23.395	.4462	
10, 9	28.000	19.102	.0046	S
2, 3	17.722	20.755	.0932	
2, 4	30.600	20.261	.0035	S
2, 5	33.400	20.261	.0015	S
2, 6	39.200	20.261	.0002	S
2, 7	50.500	20.755	<.0001	S
2, 8	-3.900	24.350	.7508	
2, 9	33.100	20.261	.0017	S
3, 4	12.878	19.625	.1953	
3, 5	15.678	19.625	.1158	
3, 6	21.478	19.625	.0324	S
3, 7	32.778	20.135	.0017	S
3, 8	-21.622	23.824	.0747	
3, 9	15.378	19.625	.1229	
4, 5	2.800	19.102	.7713	
4, 6	8.600	19.102	.3730	
4, 7	19.900	19.625	.0470	S
4, 8	-34.500	23.395	.0044	S
4, 9	2.500	19.102	.7952	
5, 6	5.800	19.102	.5474	
5, 7	17.100	19.625	.0858	
5, 8	-37.300	23.395	.0021	S
5, 9	-.300	19.102	.9751	
6, 7	11.300	19.625	.2553	
6, 8	-43.100	23.395	.0004	S
6, 9	-6.100	19.102	.5269	
7, 8	-54.400	23.824	<.0001	S
7, 9	-17.400	19.625	.0615	
8, 9	37.000	23.395	.0023	S

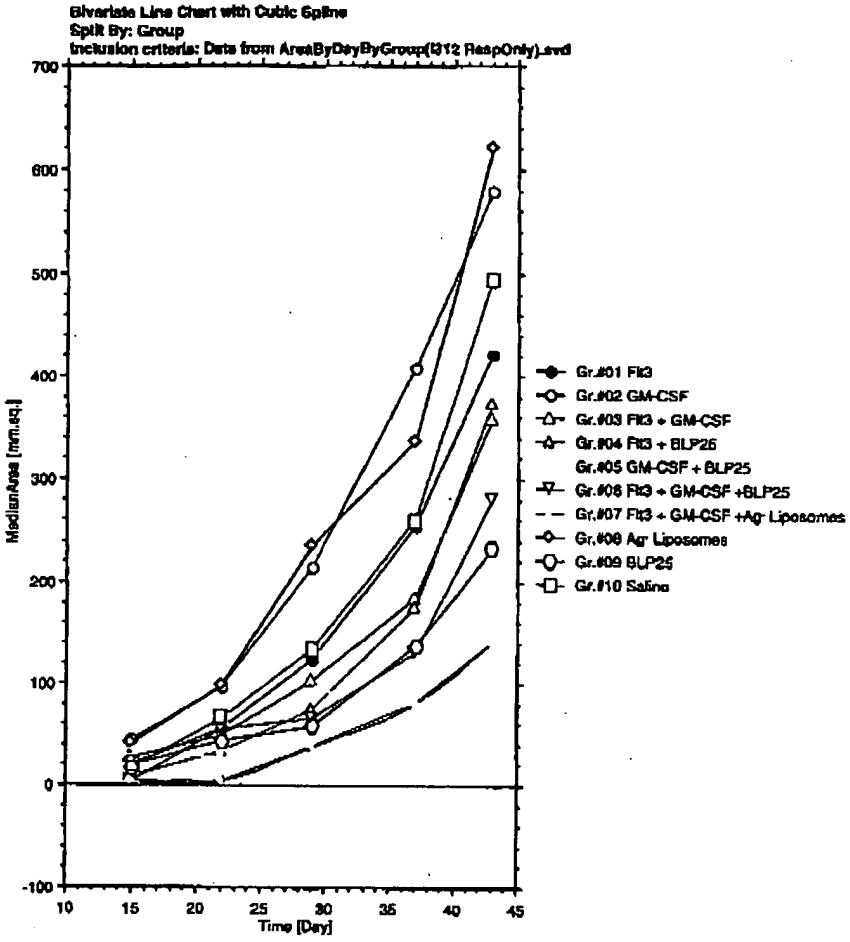

Fig 2. Tumour growth curves in mice challenged with MCA-38 MUC1 tumour and then treated with various combinations of Flt-3 ligand, pegylated GM-CSF, BLP25 liposomal vaccine or Ag liposomes. Only mice with tumours were included into this graph and in statistical evaluation.

Table 3. Statistically significant differences between mouse groups treated with various combinations of cytokines and BLP25 liposomal vaccine (only statistically significant groups are included and only mice with tumours were analyzed).

Treatment	P-value
Flt-3 + GM-CSF + Ag liposomes > Flt-3	0.0370
GM-CSF + BLP25 > Saline	0.0022
Flt-3 + GM-CSF + Ag liposomes > Saline	0.0018
BLP25 > Saline	0.0020
Flt-3 + BLP25 > GM-CSF	0.0228
GM-CSF + BLP25 > GMCSF	0.0007
Flt-3+ GM-CSF + BLP25 > GM-CSF	0.0273
Flt-3 + GM-CSF + Ag liposomes > GM-CSF	0.0006
BLP25 > GM-CSF	0.0006
Flt-3 + GM-CSF + Ag liposomes > Flt-3 + GM-CSF	0.0465
Flt-3 + BLP25 > Ag liposomes	0.0194
GM-CSF + BLP25 > Ag liposomes	0.0010
Flt-3 + GM-CSF + BLP25 > Ag liposomes	0.0221
Flt-3 + GM-CSF + Ag liposomes > Ag liposomes	0.0007
BLP25 > Ag liposomes	0.0009

ANOVA Table for RankArea(Nov02)
Inclusion criteria: Protocol I312(Tumors Only) from TumorData I312(Tumors Only).svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
GroupIndex	9	13813.285	1534.809	4.066	.0003	36.591	.894
Residual	68	25670.164	377.502				

All groups statistical analysis (only mice with tumours)

Fisher's PLSD for RankArea(Nov02)
Effect: GroupIndex
Significance Level: 5 %
Inclusion criteria: Protocol I312(Tumors Only) from TumorData I312(Tumors Only).svd

	Mean Diff.	Crit. Diff.	P-Value	
1, 10	-10.400	19.106	.2812	
1, 2	-15.500	20.066	.1279	
1, 3	2.222	19.539	.8211	
1, 4	7.125	20.066	.4810	
1, 5	17.300	19.106	.0752	
1, 6	6.167	21.570	.4526	
1, 7	24.200	22.702	.0370	S
1, 8	-19.400	22.702	.0927	
1, 9	17.600	19.106	.0704	
10, 2	-5.100	18.391	.5818	
10, 3	12.622	17.814	.1620	
10, 4	17.525	18.391	.0615	
10, 5	27.700	17.339	.0022	S
10, 6	16.567	20.021	.0686	
10, 7	34.600	21.236	.0018	S
10, 8	-8.000	21.236	.4007	
10, 9	28.000	17.339	.0020	S
2, 3	17.722	18.839	.0648	
2, 4	22.625	19.385	.0228	S
2, 5	32.800	18.391	.0007	S
2, 6	23.667	20.939	.0273	S
2, 7	39.700	22.103	.0008	S
2, 8	-3.900	22.103	.7259	
2, 9	33.100	18.391	.0006	S
3, 4	4.903	18.839	.6052	
3, 5	15.078	17.814	.0958	
3, 6	5.944	20.434	.5835	
3, 7	21.978	21.625	.0465	S
3, 8	-21.622	21.625	.0500	
3, 9	15.378	17.814	.0895	
4, 5	10.175	18.391	.2735	
4, 6	1.042	20.939	.9212	
4, 7	17.075	22.103	.1278	
4, 8	-26.525	22.103	.0194	S
4, 9	10.475	18.391	.2597	
5, 6	-9.133	20.021	.3659	
5, 7	6.900	21.236	.5189	
5, 8	-36.700	21.236	.0010	S
5, 9	.300	17.339	.9726	
6, 7	16.033	23.477	.1774	
6, 8	-27.567	23.477	.0221	S
6, 9	9.433	20.021	.3504	
7, 8	-43.600	24.521	.0007	S
7, 9	-6.600	21.236	.5372	
8, 9	37.000	21.236	.0009	S

Conclusions

1/ A significant tumour growth inhibition and a high percentage (50%) of mice without tumours suggest a therapeutic benefit when tumour bearing mice were treated with combination of both cytokines and Ag liposomes.

2/ A significant tumour inhibition was observed when mice were treated with BLP25 liposomal vaccine alone (group 9), but not when mice were treated with a combination of Flt-3 and GM-CSF (group 3). Some inhibition (significant compared to saline group and Ag liposomes) was observed when mice were treated with Flt-3 alone (group 1).

3/ When BLP25 vaccine was combined with Flt-3 and GM-CSF 4 out of 10 mice were found tumour free, however no statistically significant tumour growth inhibition was observed in the remaining mice with tumours when compared to BLP25 treatment alone.

4/ The results look encouraging and it would be worthwhile to repeat and extend this experiment.

Discussion

Flt-3 ligand and GM-CSF are known to be potent stimulators of DCs maturation and increase the number of functionally active DCs at injection sites. These cytokines seem to be good candidates to combine as adjuvants with various antigens. Our MUC1 based liposomal vaccine contains an additional adjuvant – lipid A which is known to accelerate the maturation process of DCs. We have found that incubation of human DCs with LPS or BLP25 liposomes greatly increases the percentage of class II, CD83 and CD86 molecules and stimulates anti-MUC1 T cell responses. The working hypothesis is that injections of Flt-3 and GM-CSF will increase the number of DCs which will lead to enhanced MUC1 antigen presentation, as we have found that lipid A rapidly enhances DC maturation, a synergistic effect with Flt-3 and GM-CSF might be expected. Our preliminary data seem to support this hypothesis. We have observed a high percentage of mice without tumours in two groups where mice were treated with both cytokines and BLP25 liposomal vaccine or Ag liposomes. The high percentage of mice without tumours in a group where Ag liposomes were injected can be explained by an increased number of functionally active DCs that can uptake and process tumour antigens shed from dead tumour cells. However, in all groups where vaccine or cytokines were administered separately no significant numbers of tumour "no takes" were observed. Thus, the number of tumour "no takes" in these two groups suggest a synergistic effect when combined treatment is performed. When tumour size only was analysed, again the mice in group 7 where Ag liposomes were administered together with both cytokines showed a statistically significant inhibition of tumour growth. When the cytokine or Ag liposomes were administered separately only Flt-3 induced an anti-tumour effect, but this was not as potent as the combined treatment. BLP25 vaccine alone generated an inhibition of

tumour growth but the combination with Flt-3 and GM-CSF did not improve significantly the anti-tumour effect. However, GM-CSF was administered i.p. but not s.c., and maybe some higher dose of GM-CSF should be used. All these primary observations are very promising and it would be worthwhile to repeat this experiment in order to confirm and extend these observations.